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LAMTOR2/LAMTOR1 complex is required for TAX1BP1-mediated xenophagy(Abstract_要旨)

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論文題目	LAMTOR2/LAMTOR1 complex is required for TAX1BP1 - mediated xenophagy (LAMTOR2/LAMTOR1 複合体はTAX1BP1 を介したゼノファジーを制御する)		
(論文内容の要旨)			
<p>Xenophagy, also known as antibacterial autophagy, plays a role in host defense against invading pathogens such as Group A <i>Streptococcus</i> (GAS) and <i>Salmonella</i>. In xenophagy, autophagy receptors are used in the recognition of invading pathogens and in autophagosome maturation and autolysosome formation. However, the mechanism by which autophagy receptors are regulated during bacterial infection remains poorly elucidated.</p> <p>LAMTOR2, also named p14, is a member of the rag-regulator complex. LAMTOR2 functions as a late-endosome/lysosome adaptor through LAMTOR1, a lipid raft anchor protein, and is involved in regulating endosomal biogenesis and ERK activation. Previously, LAMTOR2 was identified as one of the ligands of NBR1, which implied that LAMTOR2 might be involved in the autophagy mechanism and might also perform other unknown functions. However, no study thus far has investigated the role of LAMTOR2 in autophagy triggered in the defense against pathogen invasion. The aim of this study was to examine the function of LAMTOR2 in xenophagy and to investigate the relationship between LAMTOR2 and autophagy receptors.</p> <p>Immunofluorescence staining showed that in noninfected cells, mCherry-tagged LAMTOR2 showed cytosolic localization, but it colocalized with EmGFP-galectin 3-positive bacteria in response to GAS infection. Moreover, the study examined the localization of LAMTOR2 during <i>S. enterica Typhimurium strain LT2</i> infection and observed clear colocalization of LAMTOR2 with galectin 3-positive <i>Salmonella</i>. These results suggested that LAMTOR2 is recruited to damaged membrane debris surrounding cytosolic bacteria.</p> <p>Although LAMTOR2 knockout did not markedly affect the rate of GAS invasion into HeLa cells, the percentages of cells containing galectin 3-positive GAS were significantly decreased at 2 and 4 hr after infection. Furthermore, both the number of cells with GAS-containing autophagosomes and the average of GAS-containing autophagosomes were also lower among LAMTOR2-knockout cells than wild-type cells during GAS infection. These results suggested that the escape of GAS from endosomes to the cytoplasm by SLO is inhibited in LAMTOR2-knockout cells.</p> <p>The immunoprecipitation assay showed that EmGFP-LAMTOR2 coprecipitated with FLAG-NBR1 and further that EmGFP-LAMTOR2 also coprecipitated with FLAG-TAX1BP1 and FLAG-p62. These results suggested that LAMTOR2 can interact with three autophagy receptors. in LAMTOR2-knockout cells, the recruitment of NBR1 and TAX1BP1 to autophagosomes was markedly diminished at 2 hr and at both 2 and 4 hr after GAS infection, respectively.</p> <p>Whereas the autophagosomes detected in wild-type cells colocalized with LAMP1, the autophagosomes in TAX1BP1-knockout cells lessened the LAMP1 signal. Quantification revealed that TAX1BP1 knockout resulted in a 17.1% reduction in LAMP1-positive autophagosomes, which implied that TAX1BP1 is involved in autolysosome formation in response to GAS invasion.</p>			

<p>LAMTOR1, also named p18, attaches to the endosomal membrane surface through myristoylation and palmitoylation sites in its N-terminal region and tethers signaling complexes such as the LAMTOR2/MP1/MEK1 complex. Moreover, LAMTOR1 loss results in defective lysosome function and autolysosome formation. Immunofluorescence imaging showed endogenous LAMTOR1 was detected around SLO-deficient GAS and was colocalized with GAS- surrounding LC3. In cells lacking LAMTOR1, the recruitment of LAMTOR2 to SLO-positive GAS (cytosolic GAS) was drastically diminished, which suggested that LAMTOR2 is recruited to GAS through a LAMTOR1-dependent mechanism. In LAMTOR1-knockout cells, the recruitment of TAX1BP1 was impeded, and its colocalization with autophagosomes was markedly decreased. Immunofluorescence imaging revealed LAMP1-positive GAS-containing autophagosomes were drastically decreased in LAMTOR1 knockout cells. These results suggested that LAMTOR1 plays a role in autophagosome–lysosome fusion by recruiting LAMTOR2 and TAX1BP1.</p> <p>The study investigated the functions of LAMTOR2/LAMTOR1 in xenophagy and identified the protein as a previously unrecognized xenophagy regulator that plays an essential role in autolysosome formation by recruiting the autophagy receptor TAX1BP1 to autophagosomes. Moreover, the study also implied that the rag-regulator complex might be involved in the autophagy triggered in response to pathogen invasion.</p>
<p>論文審査の結果の要旨</p> <p>細胞内へ侵入した A 群レンサ球菌やサルモネラなどの病原性細菌に対して、宿主はオートファジーを誘導して菌を排除しようとする。一般的なオートファジーは自己成分を分解して細胞の飢餓応答や恒常性維持に機能するが、病原微生物を分解するオートファジーを特にゼノファジーと呼ぶ。ゼノファジーには、ユビキチン標識した菌を認識する「オートファジーレセプター」がオートファゴソーム形成やリソソーム融合などを制御することが知られているが、それらの詳細な制御機構は不明な点が多い。</p> <p>本研究では、オートファジーレセプターの一つ TAX1BP1 の細菌へのリクルートを制御する因子として LAMTOR1/LAMTOR2 複合体を同定した。LAMTOR1 はエンドソームに局在しており、細菌によるエンドソーム膜の傷害をきっかけに LAMTOR1 依存的に LAMTOR2 がリクルートし、オートファジーレセプターTAX1BP1 のリクルートを制御していることを明らかにした。</p> <p>また、LAMTOR2 は A 群レンサ球菌がエンドソーム膜を破壊して細胞質に侵入する過程にも関与することを示した。LAMTOR1/LAMTOR2 複合体を介してリクルートされた TAX1BP1 は、菌を取り囲んだオートファゴソームとリソソームの融合を制御しており、細胞内での殺菌寄与することを示した。今回の結果は、オートファジーレセプターの一つである TAX1BP1 の新たな制御機構を示している。</p> <p>以上の結果は、細胞内免疫を制御するオートファジーレセプターの制御機構の解明に貢献しており、細菌学や免疫学の発展に寄与するものと考えられる。</p> <p>したがって、本論文は博士（医学）の学位論文として価値あるものと認める。</p> <p>なお、本学位授与申請者は、令和元年 11 月 21 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。</p>